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# Functional C8 associated with human platelets

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## SUMMARY

Haemolytic assay for C8 revealed its association in functionally active form with washed human platelets. Platelet-bound C8 haemolytic activity was inhibited by $F(ab')_2$ anti-C8 and was undetectable in the platelet suspension obtained from three C8 deficient patients. Incubation of platelets from C8 deficient individuals in normal plasma did not restore C8 haemolytic activity, indicating that platelets do not absorb C8 from plasma *in vitro* during platelet preparation. Thrombin, a mediator of the platelet release reaction, did not induce the release of C8 from normal platelets. Conversely, lysis of EAC1-7,9 by platelet bound C8 was not accompanied by release of β-thromboglobulin or serotonin from the platelets. C8 was detected in a homogenate prepared from platelets as well as in the supernatant collected after high speed centrifugation of the homogenate. The association of C8 with platelets as an individual component rather than as part of the C5b-9 membrane-attack complex was supported by the following evidence: (a) platelet bound C8 eluted from a Sephacryl S-200 column at the same volume as C8 from normal human serum; (b) $F(ab')_2$ anti-C8, but not $F(ab')_2$ anti-C5, inhibited platelet C8 activity; (c) the platelet homogenate, which lysed EAC1-7,9, had no effect on EAC43 which are susceptible to the lytic activity of the C5b-9 complex

**Keywords** C8 functional platelets

## INTRODUCTION

A number of studies have demonstrated the association of complement proteins with platelets. (Nomenclature for the complement components in this paper conforms with that agreed upon by the World Health Organization (1968) *Bull. WHO.* **39**, 935.) Functional complement activity has been convincingly demonstrated for platelet-bound C1 (Wautier *et al.*, 1976), and more recently for factors D and H (Kenney & Davis, 1981), and C1-inhibitor (Schmaier, Smith & Colman, 1985). Other experimental work has indicated that antisera to C5, C6 or C9 inhibit both thrombin and arachidonate mediated serotonin release, suggesting that late acting complement components might be associated with the platelet surface (Polley & Nachman, 1978; Polley, Nachman & Weksler, 1981). The issue of whether these components were present as discrete, functionally active molecules or as part of membrane attack complexes C5b-9 present on the platelet surface was not addressed in these studies. This is an important consideration since an earlier report noted that

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incubation of platelets in fresh serum, even in the presence of EDTA, resulted in platelet dependent complement activation and formation of the C5b-9 complex which in turn bound to the platelets (Zimmerman & Kolb, 1976). During the evaluation of three individuals with C8 deficiency, we noted that normal platelets restored haemolytic activity to these patients' sera. Since the platelets had been washed repeatedly to remove any contamination with plasma C8, we investigated the possibility that this effect was due to C8 associated with the platelet surface.

## MATERIALS AND METHODS

*Buffers.* Tyrode's buffer was prepared as described previously (Mustard *et al.*, 1972) and was supplemented with 0·2% bovine serum albumin (BSA) and 0·03 M adenosine (Zimmerman & Spiegelberg, 1975). Veronal buffered saline (VBS) containing $Ca^{2+}$ and $Mg^{2+}$ (Mayer, 1961) was used in the haemolytic assays. Phosphate buffered saline (PBS) pH 7·4 was made up with 0·15 M NaCl and 5 mM sodium phosphate. The following buffers were used for the enzyme-linked immunoassay (ELISA): coating buffers, 0·1 M sodium carbonate-bicarbonate buffer pH 9·6; washing buffer, PBS containing 0·01% Tween-20; blocking buffer, PBS containing 1% BSA (Sigma Chemical Company, St Louis, Missouri); substrate buffer, 0·1 M citrate-phosphate buffer pH 5.

*Preparation of washed platelets, platelet homogenate, and platelet poor plasma.* Peripheral blood from normal adults was collected directly into plastic tubes containing 20 mM EDTA and immediately processed at room temperature as described previously (Zimmerman & Kolb, 1976). Briefly, the blood sample was centrifuged at 190 *g* for 15 min and the resulting platelet-rich plasma (PRP) was recentrifuged at 600 *g* for 1 min to eliminate contaminating red blood cells and leukocytes. The platelets were then pelleted at 900 *g* for 12 min, washed four times with Tyrode's buffer containing 0·2% BSA, and finally resuspended in VBS at a concentration of $10^8$ cells/ml. In some experiments, the purified platelet suspension was subjected to four cycles of freeze-thawing in order to obtain the platelet homogenate. The homogenate was centrifuged (200, 000 *g*; 45 min) to obtain the clarified supernatant which was submitted to chromatography (see below). Plasma depleted of platelets was prepared by centrifugation of PRP at 3,000 *g* for 10 min followed by recentrifugation of the recovered supernatant at 100,000 *g* for 45 min (Kontron Ultracentrifuge TGA-65; Basel, Switzerland).

*Platelet labelling and marker release.* Tritiated serotonin ($\sim$25 mCi/mmol; P-L Biochemical, Inc., Milwaukee, Wisconsin) and $^{14}C$ adenine ($\sim$50 mCi/mmol; Sigma Chemical Company, St Louis, Missouri) were employed as markers of platelet dense bodies and cytoplasm respectively. Platelet rich plasma was incubated with $^3H$ serotonin (6 $\mu$l/ml) PRP; final concentration 0·2 $\mu$M) and $^{14}C$ adenine (11 $\mu$l/ml PRP; final concentration 20 $\mu$M) for 40 min at 22°C with occasional gentle mixing (Clark & Klebanoff, 1979). Following incubation, the radioactive platelets were harvested, washed and adjusted to $1 \times 10^8$ per ml as described above. The release of $\beta$-thromboglobulin, a constituent of platelet $\alpha$-granules, was determined using a radioimmunoassay kit (Amersham, England). In these experiments reaction mixtures contained EAC1-7 plus C9 and platelets in the presence or absence of initiators or inhibitors of the platelet release reaction. The release reaction was initiated by the addition of thrombin or phorbol myristate acetate (1 unit/ml or 10 ng/ml final concentration, respectively). The effect of preincubation of the platelets with a combination of inhibitors on the platelet release reaction [2-deoxyglucose, dinitrophenol and adenosine (Sigma Chemical Company) at final concentrations of 5 mM, 50 $\mu$M, and 100 $\mu$M, respectively] on the release of platelet granule and cytoplasmic markers as well as on the lysis of EAC1-7 by intact platelets was assessed in three separate experiments. Marker release was calculated by subtracting the release from unstimulated platelets from that of stimulated platelets and expressing that value as a percentage of the total marker content of the platelets as determined by disruption in 1% Triton X-100 (Sigma Chemical Company).

*Complement reagents and haemolytic assays.* The purification of C1s, C8 and C9 and the preparation of euglobulin fractions containing either activated $\overline{C56}$ or the mixture of C7, C8 and C9 were carried out according to published procedures (Sakai & Stroud, 1973; Steckel *et al.*, 1980; Biesecker & Müller-Eberhard, 1980; Thompson & Lachmann, 1970; Lachmann & Thompson, 1970). Human sera with selective deficiencies of C3 (Tedesco *et al.*, 1986), C5 (Ross & Densen, 1984), C6 (Tedesco, *et al.*, 1981), and C8 (Tedesco *et al.*, 1980) were used for the haemolytic titration of the missing components. Sensitized sheep erythrocytes (EA), sheep EAC1-3 and EAC1-7, and guinea pig EAC1-3 were prepared as described previously (Tedesco *et al.*, 1980). Sheep and guinea pig EAC43 were prepared by allowing the corresponding intermediate EAC1-3 to decay at 37°C for 2 h in PBS containing 10 mM EDTA (Lachmann & Hobart, 1978).

In the haemolytic assays 1·0 ml of platelet suspension or platelet homogenate was mixed with 0·05 ml of target erythrocytes ($3 \times 10^8$/ml) and incubated at 37°C for 60 min. Haemolysis was determined spectrophotometrically at 415 nm. The target cells for the titration of C3, C5 and C6 were EA suspended in 1/10 C3, C5 or C6 deficient serum respectively, while guinea pig EAC1-3 in 1/10 euglobulin fraction containing $\overline{C56}$ were used for the titration of C7. Sheep EAC1-3 incubated in 1/50 C8-deficient serum were used to prepare EAC1-7 cells for assay of C8 in the presence of 20 CH50 units of purified C9, and EAC1-8 prepared with EAC1-7 and purified C8 were used for the assay of C9 (Tedesco *et al.* 1983b). The number of haemolytic sites per cell and the effective molecules of each component were calculated according to previously published methods (Rapp & Borsos, 1970). Controls for the haemolytic assays included the cell intermediates alone and EA with platelets or platelet homogenate.

*Antisera.* Specific antisera against human C1s and C8 were produced in rabbits by repeated subcutaneous injections of highly purified components in complete Freund's adjuvant. These antisera produced a single precipitin line when tested by immunodiffusion analysis against human serum and were able to inhibit specific lysis induced by the individual components (Tedesco *et al.*, 1983). Goat anti-human C5 was purchased from Meloy Laboratories (Springfield, VA). The purification of the IgG fraction from antisera to C1s, C5 and C8 by DEAE-chromatography and the preparation of the $F(ab')_2$ fragments from IgG by pepsin treatment of specific immunoprecipitates have been reported previously (Tedesco *et al.*, 1983).

*Biotin labelling of $F(ab')_2$ anti-C8 antibodies.* The biotinylation of $F(ab')_2$ antibodies was carried out using act-biotin (IBF-LKB, Milano, Italy) according to instructions of the manufacturers. Briefly, 250 μg of $F(ab')_2$ antibodies (1 mg/ml), dissolved in 0·2 M sodium bicarbonate pH 8·8 containing 0·15 M NaCl was mixed with 20 μg of act-biotin in N-dimethylformamide (0·8 mg/ml) and incubated at RT for 15 min. The reaction was stopped by the addition of ammonium chloride pH 6 at 100 mM final concentration. The biotin-labelled $F(ab')_2$ antibodies were separated from unbound act-biotin by gel filtration on Sephadex G75 (Pharmacia, Uppsala, Sweden) column (1·5 × 29 cm) equilibrated with 10 mM phosphate buffer pH 7·4 containing 0·5 M NaCl.

*Antigenic quantification of platelet-associated C8 by an antibody consumption assay.* The basic principle of this assay is to measure platelet bound C8 from the amount of specific antibodies absorbed by the platelets. This was accomplished by mixing platelet suspension varying from 2 to $6 \times 10^8$ cells/ml with biotin-labelled $F(ab')_2$ anti-C8 at 1/100 final dilution to a total volume of 0·5 ml. Preimmune IgG (50 μg/ml) obtained from the same rabbit producing the anti-C8 antiserum were added to the mixture to reduce non-specific binding of $F(ab')_2$ anti-C8 to the platelets. After incubation at 37°C for 15 min, the platelets were removed by centrifugation at 1,000 ***g*** for 15 min and the supernatant was assayed for residual antibody activity by a solid phase ELISA. This assay was carried out on 96 wells microplate M129A (Dynatech, Alexandria, VA) by coating each well with 100 μl of purified C8 (30 μg/ml) in coating buffer overnight at RT. After repeated washings with the washing buffer the remaining sites on the plastic were blocked for non-specific absorption with 100 μl of blocking buffer for 2 h at RT. The bound C8 was then reacted with 100 μl of 1/100 biotin-labelled $F(ab')_2$ anti-C8 for 1 h at RT followed by incubation with 100 μl of 1/200 avidin-peroxidase (Miles-Yeda, Torino, Italy) in PBS-BSA 1% for 30 min at RT after extensive washings. The enzymatic reaction was developed at RT by adding 100 μl of a solution of orthophenilenediamine (Sigma Chemical Company, St Louis, Missouri) at the concentration of 4 mg/10 ml substrate buffer containing 4 μl of 30% hydrogen peroxide and was blocked after approximately 5 min by the addition of 50 μl of 2·5 M $H_2SO_4$. Absorbance was read at 490 nm with Dynatech micro ELISA reader MR850. The amount of platelet absorbed antibodies was calculated with reference to a standard inhibition curve constructed by measuring the amount of $F(ab')_2$ anti-C8 absorbed by varying concentrations of purified C8.

*Gel filtration on Sephacryl S-200.* Fractionation of normal human serum or 2 ml of the clarified platelet homogenate (20 mg of protein/ml) was performed on a Sephacryl S-200 (Pharmacia, Uppsala, Sweden) column ($2{\cdot}5 \times 100$ cm) equilibrated with 10 mM phosphate buffer pH 7·4 containing 0·5 M NaCl and 2 ml fractions collected at a flow rate of 20 ml/h as described previously (Tedesco *et al.*, 1983).

*Statistical analysis.* The standard error of the mean was used as a measure of variance. Comparison of group means was performed using Student's *t*-test. $P < 0{\cdot}05$ was used as an indicator of biological significance.

## RESULTS

*Association of C8 with human platelets.* Normal platelets harvested from acid-citrate-dextrose or EDTA anticoagulated blood and incubated in the sera from three C8 deficient individuals restored haemolytic activity to these sera. C8 haemolytic activity was detectable when as few as $1{\cdot}2 \times 10^7$ platelets were used in the assay and increased to an average of 1·3 site-forming units/cell (Fig. 1). Functional titration of all the other late acting components revealed their assocation with the platelets varying from approximately 1 site-forming unit/cell for C5, C6 and C7 and 1·5 sites/cell for C9. No evidence was found for the presence of haemolytically active C3.

The possibility that plasma contaminating the platelet preparation might be the source of haemolytically active C8 was investigated using platelet-depleted plasma. Platelet-depleted plasma, prepared as described in Materials and Methods, was processed similarly to that for platelets during the washing procedure. The final volume was made equal to that of the platelet suspension prepared simultaneously from an equal volume of PRP. No C8 activity was detected in the final wash solution, indicating that functional C8 was associated with the platelets and not contributed by residual plasma contaminating the plasma preparation. These results did not exclude the possibility that C8 was absorbed by the platelets during preparation of the platelet suspension. This alternative was examined by collecting blood from each of three C8 deficient patients and a normal individual directly into two volumes of platelet-depleted $AB^+$ or C8 deficient plasma containing 20 mM EDTA. Platelets from the three C8 deficient patients did not acquire C8 haemolytic activity during suspension in $AB^+$ plasma. Conversely normal platelets did not lose C8 activity during incubation in C8 deficient plasma (Table 1).

*Localization of the active components on the platelets.* The requirement for intact platelets as a source of functional C8 was examined by measuring the C8 haemolytic activity of intact as well as

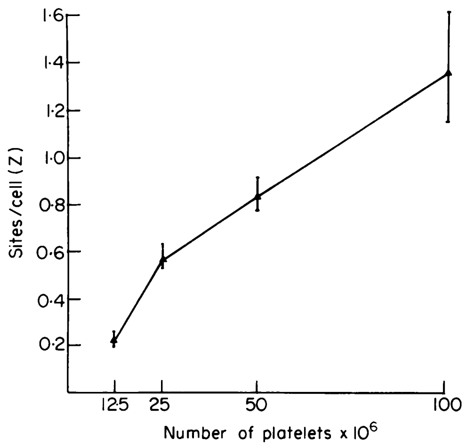


**Fig. 1.** Lysis of EAC1-7,9 induced by increasing numbers of washed human platelets expressed as the number of haemolytic sites/cell (Z). The results are reported as means with the 95% confidence limits of 10 different experiments.

**Table 1.** Platelet induced lysis of EAC1-7,9

| Incubation condition | % Lysis ($\bar{x} \pm$ s.e.m.) |
|---|---|
| Normal platelets + buffer ($n=8$) | $89 \cdot 5 \pm 2 \cdot 4$ |
| Normal platelets + C8D plasma ($n=2$) | $85 \cdot 7 \pm 8 \cdot 8$* |
| C8D platelets + buffer ($n=4$) | $10 \cdot 6 \pm 6 \cdot 1$† |
| C8D platelets + normal ($AB^+$) plasma ($n=4$) | $7 \cdot 1 \pm 1 \cdot 5$† |

* Blood drawn directly into plasma.
† $P < 0 \cdot 001$ vs normal platelets.

disrupted platelets before and after high speed centrifugation. All ($104 \pm 7$%) of the C8 activity in the platelet homogenate, was recovered in soluble form in the post 200,000 *g* supernatant of the homogenate (Table 2).

We also examined the possibility that C8 might be secreted during the platelet release reaction induced by specific stimulation or during contact with EAC1-7,9 cells. The release of serotonin, β-thromboglobulin, and adenine from unstimulated platelets in the presence of EAC1-7,9 were all less than 5·0%. However 60·7% of the serotonin and 80% of the β-thromboglobulin were discharged from the platelets upon stimulation of the platelet release reaction. Release of adenine, the cytoplasmic marker, remained unchanged under these conditions. Inhibitors of the platelet release reaction reduced granule marker release from stimulated platelets by 40%. Haemolysis of EAC1-7 cells induced by platelets alone ($91 \cdot 8 \pm 3 \cdot 0$%), was not significantly different from that induced by platelets incubated with initiators or inhibitors of the platelet release reaction ($81 \cdot 2 \pm 8 \cdot 8$%). Thus EAC1-7,9 cells did not induce the platelet release reaction, nor did haemolysis of EAC1-7,9 cells correlate with the platelet release reaction.

*Inhibition of platelet associated C8 activity by specific antiserum and immunochemical quantification of platelet-bound C8.* Further evidence for the presence of C8 on platelets were sought by examining the ability of specific antibody to inhibit the haemolytic activity of platelet associated C8. Both intact platelets and platelet homogenate were incubated with $F(ab')_2$ anti-C8 or, as control, with $F(ab')_2$ anti-C1s or buffer for 15 min at 37°C before residual haemolytic activity was assayed. As shown in Table 3, C8 activity was completely eliminated in the homogenate and, to a large extent, in intact platelets by anti-C8 but not by anti-C1s antibodies. Platelet bound C8 was also measured immunochemically as described in Materials and Methods with reference to a standard

**Table 2.** Comparison of C8 haemolytic activity in intact platelets, platelet homogenate and homogenate supernatant

| Number of platelets ($\times 10^6$) | % lysis of EAC1-7,9 by | | |
|---|---|---|---|
| | Intact platelets | Platelet homogenate | Post-200,000 *g* supernatant* |
| 12 | 22 | 8 | 9 |
| 25 | 45 | 15 | 18 |
| 50 | 59 | 36 | 34 |
| 100 | 75 | 58 | 52 |

* % Recovery (C8 activity in supernatant/C8 activity in homogenate $= 104 \pm 7$% (range 90–120%) in 1 experiment performed in triplicate.

**Table 3.** The effect of anti-C8 on platelet or platelet homogenate induced haemolysis of EAC1-7,9

| Reagents | % lysis of EAC1-7,9 |
|---|---|
| Intact PLT + buffer | 75 |
| Intact PLT + anti-C8 | 24 |
| Intact PLT + anti-C1s | 67 |
| PLT homog + buffer | 68 |
| PLT homog + anti-C8 | 2 |
| PLT homog + anti-C1s | 67 |

PLT, Platelet; homog, homogenate.

inhibition curve with purified C8 (Fig. 2). The mean amount of platelet C8 detected in two different platelet preparations using varying cell numbers was found to be 3 ng/$10^8$ platelets, which correspond to approximately 125 molecules of C8 per platelet.

*Evidence that platelet bound C8 is not part of the C5b-9 membrane attack complex.* Three types of experiments were performed to ascertain whether platelet associated C8 existed in the molecular form or as part of the C5b-9 membrane attack complex. First, the chromatographic profiles for C8 activity in normal serum and in the supernatant obtained after ultracentrifugation of the platelet homogenate were compared after molecular sieve chromatography on a Sephacryl S-200 column. Analysis of the column fractions for C8 haemolytic activity using EAC1-7,9 cells indicated that C8 in normal serum and platelet-associated C8 had an identical elution volume from the column (data not shown). Second, the ability of $F(ab')_2$ anti C8 and $F(ab')_2$ anti C5, which has been shown to block C5b-9 activity (Tedesco, Silvani & Sirchia, 1981), were examined for their ability to inhibit the C8 activity associated with platelets and the peak of C8 activity obtained by fractionation of the supernatant from the platelet homogenate on the Sephacryl S-200 column. $F(ab')_2$ anti C8 reduced haemolysis of EAC1-7,9 by platelets and the fractionated supernatant from 70% to less than 2% while $F(ab')_2$ anti C5 was without effect (Fig. 3). Third, we assayed the platelet homogenate for

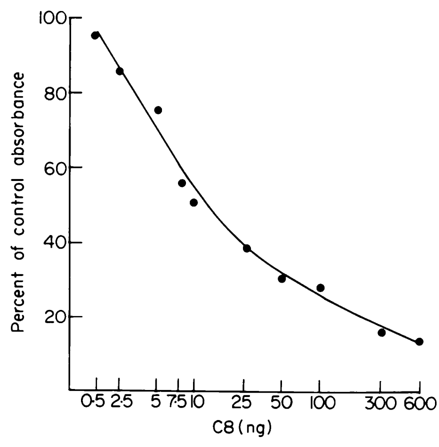


**Fig. 2.** Inhibition curve of binding of biotin-labelled $F(ab')_2$ anti-C8 obtained with purified C8. Increasing amounts of C8 (abscissa) were incubated with biotin-labelled $F(ab')_2$ anti-C8 used at 1/100 final dilution to a total volume of 0·5 ml for 15 min at 37°C. The residual antibody activity was measured by ELISA as indicated in Materials and Methods. The absorbance readings at 490 nm are reported on the ordinate as percent values of the control sample [$F(ab')_2$ anti-C8 + buffer].

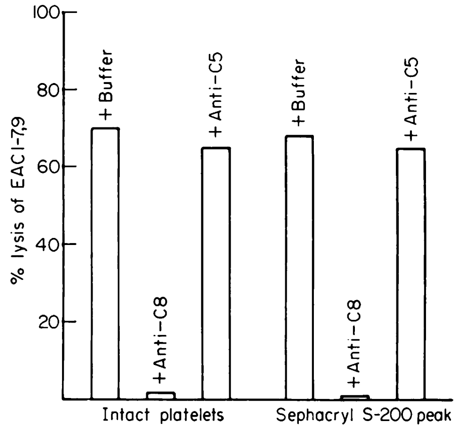


**Fig. 3.** Effect of F(ab′)$_2$ anti-C8 and F(ab′)$_2$ anti-C5 on the haemolysis of EAC1-7,9 induced by intact platelets or the haemolytically active C8 peak obtained upon fractionation of the platelet homogenate on a Sephacryl S-200 column. One ml of platelet suspension ($3 \times 10^8$ cells) or the haemolytically active peak obtained from an equivalent number of platelets was incubated with 50 $\mu$l of F(ab′)$_2$ antibody fragments (1 mg/ml) or buffer for 15 min at 37°C prior to mixing with EAC1-7,9.

haemolytic activity using C3b-coated red cells, which are susceptible to the haemolysis by the C5b-9 complex. These cells were prepared using erythrocytes from a variety of sources as described in Materials and Methods. Neither of the EAC43 intermediates prepared using sheep or guinea pig red cells were lysed by the platelet homogenate. In contrast, both were lysed by the C5b-9 complex prepared by mixing the euglobulin containing $\overline{C56}$ with the euglobulin containing C7, C8 and C9. These results indicate that the lysis of EAC1-7,9 cells is dependent on C8 and not C5b-9 transferred intact from the platelet surface since under the latter conditions lysis of EAC43 cells comparable to the positive control would have been expected. In addition, the failure of EAC43 cells to lyse in the presence of the platelet homogenate is strong evidence against the possibility that haemolysis was mediated by proteolytic enzymes released from the platelets during preparation of the homogenate.

## DISCUSSION

The results presented here are consistent with the assocation of C8 with human platelets in functionally active form, as shown by the detection of this component in the platelet suspension using a specific haemolytic assay. The possibility that both intact platelets and platelet homogenate exert a nonspecific haemolytic effect on sensitized sheep erythrocytes through the action of proteolytic enzymes is discounted by the fact that EA were not lysed in control experiments and haemolytically active C3 was undetectable in the platelet suspension (Kenney & Davis, 1981). Moreover, the inhibition of C8 haemolytic activity in the platelet homogenate by F(ab′)$_2$ anti-C8, but not by F(ab′)$_2$ anti-C1s, further emphasizes the specificity of these results.

Although each of the late acting complement components from C5 to C9 is detected in haemolytically active form in the platelet suspension, functional C8 appears to be present as a molecular entity rather than as part of C5b-9 complex on platelets. Evidence supporting this conclusion was derived from the observations that (1) platelet-associated C8 and C8 in normal human serum have the same elution volume from a Sephacryl S-200 column; (b) F(ab′)$_2$ anti-C5, unlike F(ab′)$_2$ anti-C8, did not inhibit the C8 haemolytic activity of the platelet supernatant as would be expected if C8 were present as part of the C5b-9 complex; (c) none of the EAC43 erythrocyte intermediates, which are susceptible to the lytic activity of the C5b-9 complex, were lysed by the platelet homogenate. It should be noted that the assays used here were designed to detect functionally active C8 or the C5b-9 complex and do not exclude the presence of functional inactive but antigenic C5b-9 complexes reported previously (Zimmermann & Kolb, 1976).

The nature and the site of the association of C8 with platelets are incompletely defined by the present experiments. The fact that platelets from C8 deficient patients do not acquire C8 during suspension in plasma from individuals mitigates against the possibility that binding of C8 in an in-vitro artifact. The failure of repeated washes to remove C8 from the platelets suggests that the association is specific, as it has been shown for the binding of other plasma proteins to platelets (Nachman & Harpel, 1976; Zucker *et al.*, 1979). As noted by others (Kenney & Davis, 1981), the fact that not all plasma proteins are found in association with platelets further supports the specificity of this association and suggests that the results cannot be accounted for by simple, nonselective mechanisms. Indirect evidence suggests that C8 is bound to the platelet surface and not located within platelet granules, since inhibitors of the platelet release reaction have no effect on the haemolysis of EAC1-7,9 cells. A tight association of functional C8 with platelets is unlikely considering that all of the haemolytic C8 in the platelet homogenate could be recovered in the supernatant after high speed centrifugation. However, this conclusion may not be true for the majority of platelet bound C8 molecules, which can be detected in a more substantial number by the ELISA than by the haemolytic assay. Whether the greater part of immunochemically detectable C8 on platelets is haemolitically inactive or is incapable of exerting haemolytic activity because of a tighter association with platelets needs to be clarified.

The amounts of C8 and of each of the late acting components associated with platelets are of the same order of magnitude as those observed for C1 (Wautier *et al.*, 1976) and factor D (Kenney & Davis, 1981). The low concentration of platelet-bound terminal components compared to their plasma levels suggests that these components may not play a significant role in the circulation. Conversely, platelets accumulating in a substantial number at the inflammatory site may represent an important vehicle of the late acting components, potentially lytic for susceptible targets. At any rate, a more precise definition of the functional importance of platelet associated C8 and other complement components in host defence requires further studies.

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## REFERENCES

BIESECKER, G. & MULLER-EBERHARD, H.J. (1980) The ninth component of human complement: purification and physicochemical characterization. *J. Immunol.* **124,** 1291.

CLARK, R.A. & KLEBANOFF, S.J. (1979) Myeloperoxidase-mediated platelet release reaction. *J. Clin. Invest.* **63,** 177.

KENNEY, D.M. & DAVIS, A.E. (1981) Association of alternative complement pathway components with human blood platelets: Secretion and localization of factor D and $\beta$1H globulin. *Clin. Immunol. Immunopath.* **21,** 351.

LACHMANN, P.J. & HOBART, M.J. (1978) Complement technology. In: *Handbook of Experimental Immunology* (3rd ed), Vol. 1. (ed. by D.M. Weir) 5A.1. Blackwell Scientific, London.

LACHMANN, P.J. & THOMPSON, R.A. (1970) Reactive lysis: The complement-mediated lysis of unsensitized cells. II. The characterization of activated reactor as $\overline{C56}$ and the participation of C8 and C9. *J. Exp. Med.* **131,** 643.

MAYER, M.M. (1961) Complement and complement fixation. In: *Experimental Immunochemistry* (2nd Ed) (ed. by Elvin A. Kabat & Manfred M. Mayer) p. 133. Charles C. Thomas, Springfield.

MUSTARD, J.F., PERRY, D.W., ARDLIE, N.G. & PACKHAM, M.A. (1972) Preparation of suspensions of washed platelets from humans. *Brit. J. Haematol.* **22,** 193.

NACHMAN, R.L. & HARPEL, P.C. (1976) Platelet $\alpha_2$-macroglobulin and $\alpha_1$-antitrypsin. *J. Biol. Chem.* **251,** 4514.

POLLEY, M.J. & NACHMAN, R. (1978) The human complement system in thrombin-mediated platelet function. *J. Exp. Med.* **147,** 1713.

POLLEY, M.J., NACHMAN, R.L. & WEKSLER, B.B. (1981) Human complement in the arachidonic acid transformation pathway in platelets. *J. Exp. Med.* **133,** 257.

RAPP, H.J. & BORSOS, T. (1970) *Molecular Basis of Complement Action.* p. 111. Appleton-Century-Crofts, New York.

ROSS, S.C. & DENSEN, P. (1984) Complement deficiency states and infection: epidemiology, pathogenesis and consequences of Neisserial and other infections in an immune deficiency. *Medicine*, **63**, 243.

SAKAI, K. & STROUD, R.M. (1973) Purification, molecular properties and activation of C1 proesterase, C1s. *J. Immunol.* **110**, 1010.

SCHMAIER, A.H., SMITH, P.M. & COLMAN, R.W. (1985) Platelet C1 inhibitor. A secreted alpha-granule protein. *J. Clin. Invest.* **75**, 242.

STECKEL, E.W., YORK, R.G., MONAHAN, J.B. & SODETZ, J.M. (1980) The eighth component of human complement. Purification and physicochemical characterization of its unusual subunit structure. *J. Biol. Chem.* **255**, 11997.

TEDESCO, F., BARDARE, M., GIOVANETTI, A.M. & SIRCHIA, G. (1980) A familial dysfunction of the eighth component of complement (C8). *Clin. Immunol. Immunopath.* **16**, 180.

TEDESCO, F., DENSEN, P., VILLA, M.A., PETERSEN, B.H. & SIRCHIA, G. (1983) Two types of dysfunctional eighth component of complement (C8) molecules in C8 deficiency in man. Reconstitution of normal C8 from the mixture of two abnormal C8 molecules. *J. Clin. Invest.* **71**, 183.

TEDESCO, F., SILVANI, C.M., AGELLI, M., GIOVANETTI, A.M. & BOMBARDIERI, S. (1981) A lupus-like syndrome in a patient with deficiency of the sixth component of complement. *Arthritis Rheum.* **24**, 1438.

TEDESCO, F., SILVANI, C.M. & SIRCHIA, G. (1981) Effect of complement activation on granulocyte adherence to nylon fibers: Inhibitory activity of C567 complex. *Clin. Immunol. Immunopath.* **18**, 176.

TEDESCO, F., TOVO, P.A., TAMARO, G., BASAGLIA, M., PERTICARI, S. & VILLA, M.A. (1985) Selective C3 deficiency due to C3 nephritic factor factor in an apparently healthy girl. *La Ricerca Clin. Lab.* **15**, 323.

THOMPSON, R.A. & LACHMANN, P.J. (1970) Reactive lysis: The complement-mediated lysis of unsensitized cells. I. The characterization of the indicator factor and its identification as C7. *J. Exp. Med.* **131**, 629.

WAUTIER, J.L., TOBELEM, G.M., PELTIER, A.P. & CAEN, J.P. (1976) C1 and human platelets. II. Detection by immunological methods and role. *Immunology*, **30**, 459.

ZIMMERMAN, T.S. & KOLB, W.P. (1976) Human platelet-formation and uptake of the C5-9 complex of human complement. *J. Clin. Invest.* **57**, 203.

ZIMMERMAN, T.S. & SPIEGELBERG, H.L. (1975) Pneumococcus-induced serotonin release from human platelets. Identification of the participating plasma/serum factor as immunoglobulin. *J. Clin. Invest.* **56**, 828.

ZUCKER, M.B., MOSESSON, W.M., BROEKMAN, M.J. & KAPLAN, K.L. (1979) Release of platelet fibronectin (cold-insoluble globulin) from alpha granules induced by thrombin or collagen: lack of requirement for plasma fibronectin in ADP-induced platelet aggregation. *Blood*, **54**, 8.